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The Effect of *TrigonellaFoenum Graecum* (Hulbah) Seeds Powder on Lipids Profile in Cholesterol Fed Rats

By

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DEDICATION

ı`m glad to dedicate this work to:

My father…….

My mother…..

My sisters and brothers…..

My friends…….

With respect

El-rasheed

ACKNOWLEDEMENTS

Thanks to god allah the lord of the creation and the compassionate who give me mind and made this work possible.

Special grateful thanks and appreciation to my supervisor dr. Barakat Elhussien Mohammed for his patience guidance and valuable advices during the period of this work and the teaching staff of the Biochemistry department, faculty of veterinary medicine.

The endless thanks are due to my family and my friends.

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ASTRACT

The aim of the study was to evaluate the effect of the *trigonella foenum garaecum* seeds powder (TSP) on lipid profile in cholesterol fed rat

Twenty Wistar albino rats were divided into four groups named A, B, C and D of five rats each. Group A was given the basal diet and served as control, group B was given basal diet and 1% cholesterol added to the basal diet, group C was given basal diet and 1% cholesterol and 4% TSP added to the basal diet and group D was given basal diet and 1% cholesterol and 8% TSP added to the basal diet for four weeks.

Blood samples were collected from group A and B after two weeks so as to confirm the induction of hypercholesterolemia. The level of total cholesterol and LDL-c was significantly (P› 0.05) increased in group B compared to the control whereas, the level of HDL-c were significantly (P› 0.05) decreased in group B compared to the control.

Blood samples were collected from all groups after four weeks. The level of total cholesterol and LDL-c was significantly (P› 0.05) decreased in group C and D compared to group B. while the level not significantly different in group C, and D compared to group B. the level of HDL-c were significantly (P› 0.05) decreased in group C and D compared to B. there was non- significant difference in the levels of total cholesterol, LDL-c, HDL-c and triglycerides in group C and D compared to the control group.

الملخص العربي

هدفت هذه الدراسة لتقيم بذور الحلبه المسحونة علي مستوي الدهون في الفئران المغذيه إضافيا بالكولسترول.

عشرون فأرا قسموا الي أربع مجموعات أ، ب، ج و د. كل مجموعه تحتوي علي خمس فئران. حيث كانت المجموعه (أ) هي مجموعه التحكم و أعطيت الوجبة الأساسية، المجموعة (ب) أعطيت الوجبة الأساسية بإضافة 1% كوليسترول، المجموعة (ج) أعطيت الوجبة الأساسية بإضافة 1% كوليسترول و بذور الحلبة المسحونة ينسبة 4% اما المجموعة (د) أعطيت الوجبة الأساسية بإضافة 1% كوليسترول و بذور الحلبة المسحونة ينسبة 8% لمدة أربعة اسابيع.

أخذت عينات من مصل الدم بعد أسبوعين من بدايه المعالجة من المجموعة (أ) و المجموعة (ب). مستويات الكوليسترول الكلي وكوليسترول اللابوبروتين منخفض الكثافة زادت زيادة معنوية ($P< 0.05$) في المجموعة (ب) بالمقارنة مع مجموعة التحكم، بينما كوليسترول اللايبو بروتين عالي الكثافة إنخفض إنخفاضا معنويا في($P< 0.05$) المجموعة (ب) بالمقارنة مع مجموعة التحكم.

أخذت عينات من مصل الدم بعد أربعة أسابيع من كل المجموعات. مستويات الكوليسترول الكلي وكوليسترول اللابوبروتين منخفض الكثافة زادت زيادة معنوية ($P< 0.05$) في المجموعة (ج) و (د) بالمقارنه مع المجموعة (ب)، بينما لم يكن هناك فرق معنوي في مستوي الجليسريدات الثلاثية في المجموعتين (ج) و (د) بالمقارنة مع المجموعة (ب)، مستوي كوليسترول اللايبو بروتين عالي الكثافة زاد معنويا ($P< 0.05$) في المجموعتين (ج) و (د) بالمقارنة مع المجموعة (ب)، في مستويات الكوليسترول الكلي وكوليسترول اللابوبروتين منخفض الكثافة و كوليسترول اللايبو بروتين عالي الكثافة و الجليسريدات الثلاثية لم هناك فرق معنوي في المجموعتين (ج) و (د) بالمقارنة مع مجموعة التحكم.

INTRODUCTION

Lipids play many roles in biological systems. Like carbohydrates they are an important source of energy. In addition, they are essential components of membranes, they function as hormones, and they can serve as padding and thermal insulator. Lipids are complex and heterogeneous groups of compounds that they defy rational classification. However, despite their difference in structure, they share the property of being insoluble in water. Indeed they are so insoluble that they require special water-soluble lipid-protein complex (lipoprotein) for transport in the blood stream (Murray *et al*., 2003).

Dyslipidaemia, which can range from hypercholesterolemia to hyper lipoproteinemia, is one of the many modifiable risk factors for coronary artery disease (CAD), stroke and peripheral vascular disease (Chong and Bachenheimer, 2000). Atherosclerosis is a disease of the intima of large and medium-sized arteries. Prominent among resultant intimal changes is the focal accumulation of lipid, particularly cholesterol and cholesteryl esters, in fibro fatty plaques known as atheromas (WHO, 1958). The atheromas narrow the arterial lumen, damage the underlying media and frequently become ulcerated and/or calcified, thus further narrowing the arterial lumen. In humans, this disease most commonly affects the abdominal aorta, coronary, iliac, femoral and cerebral arteries (Wissler, 1980).

The use of spices as food additive has been practiced widely since ancient times. A part from enhancing the taste and flavour of food, spices have been widely believed to exert digestive stimulant action. A few medicinal properties of spices such as tonic, carminative, diuretic, and antispasmodic effects have long been recognized. These attributes are largely empirical, nevertheless efficacious, have earned their pharmacological applications in the indigenous systems of medicine as digestive stimulants and to relieve digestive disorders (Petit et al., 1995).

Fenugreek (*Trigonella foenum graecum*) natively known as Hulabah is one of the oldest medicinal plant, originating in India and Northern Africa. As a seasonal plant, fenugreek grows to an average height of two feet. The leaves and seeds, which mature in long pods, are used to prepare extracts or powders for medicinal uses (Morcos *et al.*, 1981).

Fenugreek seeds have been shown to have hypoglycaemic and anticholesterolemic actions (Sharma, 1996). Fenugreek fed to rats of both sex at dietary doses of 0%, 1%, 5% and 10% in a pure diet had no effect on either the daily food intake or growth (Muralidhara *et al.*, 2000).

There are many chemical drugs such as: statins, ezetimibe and nicotinic acid that lower blood cholesterol level but are most expensive and have many undesirable effects (Thomas, 2003). Many herbal plants that lower cholesterol concentration were studied by Prasanna (2000), El- Dakhakhny *et al.* (2000) and Sen and Bhattacharyya (2001).

Parallel with recent increasing interest in alternative/herbal medicine for the prevention and treatment of various illnesses including hypercholesterolemia and because fenugreek is used daily by many Sudanese people with tea, therefore the aim of this study is to evaluate the effect of feeding *Trigonella foenum graecum* seeds powder (TSP) mixed diet on the level of serum lipid profile in an induced hypercholesterolemic Wistar albino rats. The parameters to be measured include serum total cholesterol, low density lipoprotein cholesterol (LDL-c), high density lipoprotein cholesterol (HDL-c) and triacylglycerol.

CHAPTER ONE

LITERATURE REVIEW

1.1. Lipids:

Plasma lipids are derived from food (exogenous) or are synthesized in the body (endogenous). Lipids are relatively insoluble in water; they are carried in body fluids as soluble protein complexes known as lipoproteins (Murray *et al*., 2003).

Four main classes of lipids can be recognized, from a metabolic stand point. These are cholesterol and its esters, triglycerides, phospholipids and fatty acids (Forrester *et al.,* 1987). An understanding of the pathophysiology of plasma lipid metabolism is based on the concept of lipoproteins, the form in which lipids circulate in plasma. The principle functions of lipids are to act as energy stores and to serve as important structural component of cells. To fulfill these functions, lipids have to be transported in plasma from one tissue to another, from the intestine or the liver to other tissues such as muscular or adipose tissue, or from the other tissues to the liver (Bishop *et al.,* 2000). There are complex mechanisms that control the release of lipids from tissues into plasma, and the uptake of lipids by the tissues from the plasma. Abnormalities of these mechanisms may be associated with the development of disease, particularly ischemic heart disease (Champe and Harvey, 1994).

1.1.1 Cholesterol:

Cholesterol is a lipid found in the cell membrane of all animal tissues, and it is transported in the blood plasma of all animals. Cholesterol is also a sterol (a combination of steroid and alcohol). Because cholesterol is synthesize by all eukaryotes. Most of the cholesterol is synthesized by the body and some has dietary origin. Cholesterol is more abundant in tissues which either synthesize more or have more abundant densely-packed membrane, for example, the liver, spinal cord and brain. It plays a central role in many biochemical processes, such

as the composition of cell membrane and the synthesis of steroid hormones (Stryer, 1995).

Cholesterol from the liver and intestine is transported in plasma. About 75% esterified with fatty acids and the rest unesterified. It is taken up from plasma by different tissues. Its main route of metabolism is to bile acids, which are secreted into bile as conjugated with glycine or taurine. Unesterified cholesterol is also secreted into bile, and both undergo an entero-hepatic-circulation with some loss of cholesterol and bile acids occur daily in the faeces. Unlike that of triglycerides, plasma concentration of cholesterol does not rise after a fatty meal (Johnson *et al.,* 1991).

Since cholesterol is insoluble in blood, it is transported in the circulatory system within lipoproteins. Complex spherical particles which have an exterior composed mainly of water-soluble proteins; fats and cholesterol are carried internally (Brunzell *et al*., 2008).

1.1.2. Lipoproteins:

Lipoproteins are complexes of lipids and proteins held together by non-covalent bonds. The core is of insoluble (non-polar) cholesterol esters and triglycerides, surrounded by proteins (known as apolipoproteins), phospholipids and free cholesterol with their water soluble (polar) groups facing outwards. Clinically, the most important are the lipoproteins of the plasma, which function as major transporters of lipids. Lipoproteins are classified based on their density to five fractions including high density lipoproteins (HDL), low density lipoproteins (LDL), intermediate density lipoproteins (IDL), very low density lipoproteins (VLDL) and chylomicrones (Davidson and Sittman, 1994).

Chylomicrones are derived from the intestinal absorption of triglycerides. The VLDL is synthesized in the liver for exportation of triglycerides to the extra-hepatic tissues; LDL is the final stage in the catabolism of VLDL; IDL is a transient lipoprotein formed during the conversion of VLDL to LDL, it contains both triglycerides and cholesterol, IDL is usually undetectable in normal plasma

(Zilva *et al*., 1994). HDL is involved in VLDL and chylomicrones metabolism and also in the transport of cholesterol to the liver (Murry *et al*., 2003).

1.1.2.1 Low-density lipoprotein (LDL):

Low-density lipoprotein (LDL) is a lipoprotein that transports cholesterol and triglycerides from the liver to peripheral tissues. LDL also regulates cholesterol synthesis at these sites. It commonly appears in the medical setting as part of a cholesterol blood test, and since high levels of LDL-cholesterol can signal medical problems like cardiovascular disease, it is sometimes called "bad cholesterol". Each native LDL particle contains a single apolipoprotein B-100 molecule (Apo B-100, a protein with 4536 amino acid residues) that circulates the fatty acids, keeping them soluble in the aqueous environment. In addition, LDL has a highly-hydrophobic core consisting of polyunsaturated fatty acid known as linoleate and about 1500 esterified cholesterol molecules (Segrest *et al.,* 2001). Conditions with elevated concentrations of oxidized LDL particles, especially "small dense LDL" (sdLDL) particles, are associated with atheroma formation in the walls of arteries, a condition known as atherosclerosis, which is the principal cause of coronary heart disease and other forms of cardiovascular disease (Lewington *et al.,* 2007).

1.1.2.2. High-density lipoprotein (HDL):

HDL is the smallest of the lipoproteins. It is involved in the transport of cholesterol from the peripheral tissues to the liver. They are the densest because they contain the highest proportion of protein. They contain the A class of apolipoproteins. The liver synthesizes these lipoproteins as complexes of apolipoproteins and phospholipid, which resemble cholesterol-free flattened spherical lipoprotein particles. They are capable of picking up cholesterol, carried internally, from cells they interact with. A plasma enzyme called lecithin-cholesterol acyltransferase (LCAT) converts the free cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol) which is then sequestered into the core of the lipoprotein particle eventually making the newly synthesized HDL

spherical (Kwiterovich, 2000). They increase in size as they circulate through the bloodstream and incorporate more cholesterol molecules into their structure. High concentrations of functional HDL, which can remove cholesterol from cells and atheroma, offer protection against atherosclerosis hence termed good cholesterol (Durrington, 2003).

1.1.3 Triglycerides:

Triglycerides are fatty acid esters of glycerol, each containing three fatty acids. They are transported as lipoproteins from the intestine and the liver to various tissues, such as adipose tissue. Following hydrolysis, fatty acids are taken up, re-esterified and stored as triglycerides. Plasma triglyceride concentrations rise after a fatty meal and remain increased for several hours (Stein and Gary, 1994). Triglycerides are absorbed from the jejunum and transferred into the intestinal lymph and then to the systemic circulation. Liver and intestine are also the major sites of triglycerides synthesis. Those formed in the liver, normally being secreted into plasma, and those in adipose tissue are either stored locally or reconverted to fatty acids and glycerol prior to re-entry into the circulation. They are important storage forms of energy (Whitby *et al*., 1987).

1.1.4. Atherosclerosis and coronary heart disease (CDH):

According to the lipid hypothesis, abnormally high cholesterol levels (hypercholesterolemia), or, more correctly, higher concentrations of low density lipoprotein cholesterol (LDL-c) and lower concentrations of functional high density lipoprotein cholesterol (HDL-c) are strongly associated with cardiovascular disease because these promote atheroma development in arteries (atherosclerosis). This disease process leads to myocardial infarction (heart attack), stroke and peripheral vascular disease. Since higher blood LDL-c, especially higher LDL-c particle concentrations and smaller LDL-c particle size, contribute to this process more than the cholesterol content of the LDL-c particles (Brunzell, 2008). LDL-c particles are often termed "bad cholesterol" because they have been linked to atheroma formation. On the other hand, high concentrations of functional HDL-c, which can remove cholesterol from cells

and atheroma, offer protection. These balances are mostly genetically determined but can be changed by body build, medications, food choices and other factors (Durrington, 2003).

1.2. Hypolipidemic agents:
1.2.1. Drugs:
1.2.1.1. Statins:

Most current therapeutic approaches seek to lower LDL-c. The discovery of statins was a major milestone in lipid lowering therapy. Statins are competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, the rate limiting enzyme that catalyzes the conversion of HMG CoA to mevalonate in the liver cells. Mevalonate is the precursor molecule for cholesterol, Coenzymes Q and squalene (an intermediate in cholesterol synthesis). The decrease in the intracellular levels of cholesterol induces a higher surface expression of LDL receptors which consequently increases the clearance of plasma LDL-c. IDL and VLDL remnants are removed as well, contributing to lowering triglyceride-rich lipoprotein levels (Anonymous, 2002).

The new member, Rosuvastatin is reported to be the most potent, reducing LDL-c levels by up to 65% (in a dose range of 20-80 mg/day) in clinical studies (Olsson, *et al*., 2001). Statins also have moderate effects on HDL-c, raising levels by approximately 5%, and decrease triglyceride concentrations to a maximum of about 30% (Vega and Grundy, 1990).

Unfortunately, the mechanism of action of statins through inhibition of the mevalonate pathway inhibits the biosynthesis of vital biochemical products of this loop, including coenzyme Q10 (CoQ10). In humans, CoQ10 or ubiquinone (2,3-dimethoxy-5- methyl-6-decaprenyl-1,4-benzoquinone) is a major participant in electron transfer during oxidative phosphorylation in the mitochondria, a potent antioxidant and free radical scavenger, and a membrane stabilizer that preserves cellular integrity. These functions are particularly relevant to cardiovascular health, leading to the logical conclusion that patients on long-term statin therapy should receive supplemental CoQ10 (Bliznakov, 2002).

Besides statins, other current lipid-altering agents that lower LDL-c primarily through increased hepatic LDL receptor activity include bile acid sequestrants / resins and cholesterol absorption inhibitors such as ezetimibe. Natural approaches such as plant stanols/sterols, polyphenols, as well as nutraceuticals such as oat bran, psyllium and soy proteins are also reported to lower LDL-c (Bays and Stein, 2003).

1.2.2. Plants:

A plant-based diet that is rich in fruit, vegetables, legumes and low in saturated fat, along with regular aerobic exercise program, is a typical prescription for anyone with elevated risk of cardiovascular disease. In addition, there are a few herbs available that provide some help for persons with hyperlipidemia (Winston, 1999).

1.2.2.1. Sunflower seeds:

Rats fed the sunflower seed protein fraction showed a similar body weight gain and food efficiency ratio as rats fed casein. However, the different dietary proteins had different effects on plasma total cholesterol and triglyceride contents. The rats fed the sunflower seed protein fraction showed a significant decrease in plasma cholesterol ($p < 0.02$) and triglyceride ($p < 0.02$) concentrations compared to the casein-fed rats. These results demonstrated the hypolipidemic action of the sunflower seed protein fraction and that it can be considered as a suitable edible protein like casein. This study and others established an inverse relationship between quantity of dietary protein and blood cholesterol. This relationship suggests that cholesterol metabolism may depend on adequate protein or that cholesterol synthesis is accelerated in protein deficiency (Sen and Bhattacharyya, 2001).

1.2.2.2. Green tea:

Several epidemiologic studies have suggested that drinking either green or black tea may lower blood cholesterol concentrations and blood pressure, thereby providing some protection against cardiovascular disease. When rats were fed

green tea polyphones, blood cholesterol concentrations declined in hypercholesterolemic animals and blood pressure decreased in spontaneously hypertensive animals (Dreosti, 1996). Potential mechanisms include reduced micellar solubility and intestinal absorption of cholesterol increased fecal excretion of fat and cholesterol, reduced hepatic cholesterol concentration and up-regulation of the LDL receptor in liver cells (Bursill,. *et al,*.2001).

1.2.2.3. *Coriandrum sativum*:

In the biphasic model of triton-induced hyperlipidemia, *Coriandrum sativum* at a dose of 1g/kg body weight (Bwt) reduced cholesterol and triglycerides levels in both synthesis and excretory phases in rats, and the results were comparable with that of Liponil, a commercially available herbal hypolipidemic drug. The results suggest that coriander decreases the uptake and enhances the breakdown of lipids (Lal *et al*., 2004). The administration of 8% of *Coriandrum sativum* fruits to Wistar albino rats resulted in a significant reduction of total and LDL-c levels whereas the level of HDL-c were reported to be significantly elevated. These findings are due to the high fiber and unsaturated/ saturated fatty acids ratio content of the fruits (Shihab Eldin, 2007).

1.2.2.4. *Nigella sativa*:

Nigella sativa-treated rats had lower fasting plasma levels of cholesterol and triglycerides, and higher HDL-c as compared to pair-fed controls. The hypocholesterolemic function of *Nigella sativa* is either by reducing the synthesis of cholesterol by hepatocytes or by decreasing its fractional reabsorption from the small intestine (Ali *et al*., 2004).

1.2.2.5. Gum Arabic:

Gum Arabic is a water-soluble non-starch polysaccharides, and as such belongs to a group of compounds known to have the potential to lower plasma cholesterol in humans and to be fermented by the large bowel micro-flora (Topping, 1992). How gum arabic lowers plasma lipids is still unclear, but the mechanism is related possibly to increased fecal bile acid and neutral sterol excretion or a modification of lipid digestion and absorption (Eastwood, 1992).

1.2.2.6. Garlic (*Allium sativum* L.):

The administration of 4% and 8% of fresh crushed garlic bulbs to Wistar albino rats resulted in a significant reduction of total, LDL-c and triacylglycerol levels whereas the level of HDL-c were reported to be significantly elevated. (Maha, 2006). Regular use of garlic can be effective in reducing the risk of heart attack and stroke because it lowers total and LDL-c and triacylglycerol concentrations without affecting HDL-c concentrations (Warshafsky *et al*., 1993). Garlic is reported to decrease serum cholesterol, LDL-c and triglycerides, possibly through inhibition of HMG-CoA reductase activity and increases the excretion of cholesterol as bile acids (Kleijnen *et al*., 1989).

1.2.2.7. flaxseed (*Linum usitatissimum*):

Flour derived from flaxseed (*Linum usitatissimum*) is popular for use in bread and bakery products. It provides a nutty flavor and also increases the nutritional and health benefits of the final product. Flaxseed consumption may lower both total- and LDL-c concentrations because of its low–saturated fat content, high polyunsaturated fat, phytosterol content and mucilage content (Cunnane *et al*., 1993).

1.2.2.8. Cinnamon:

Cinnamon significantly reduced the triglycerides levels in diabetic individuals. This effect of cinnamon is particularly important for hyperlipidemic individuals. The lipid lowering effect of cinnamon might be due to insulin potentiating action of cinnamon. Usually, when glucose metabolism is improved, lipid metabolism is also improved. Some constituents of cinnamon are blocking the synthesis of cholesterol or facilitating the clearance of cholesterol from the body. The insulin potentiating property of cinnamon may help to reduce cholesterol level. High fibers amount present in cinnamon decrease fat absorption by the gut resulting in a decrease level of triglycerides in the blood (Alam *et al.*, 2003).

1.2.2.9. *Zingiber officinale Roscoe*:

In rabbits fed high cholesterol diets, ginger extracts had antilipemic effects, reducing atherogenesis and high lipid levels (Bhandari *et al*., 1998). However, in hypercholesterolemic rats, the data on ginger's effects has been conflicting. Some studies reported positive effects and others found no effects (Sambaiah and Srinivasan, 1991). In experimental mice, ginger significantly impaired cholesterol biosynthesis and lowered serum cholesterol concentrations (Tanabe *et al*., 1993).

1.2.2.10. *Trigonella foenum graecum* (fenugreek):

The seeds of *Trigonella foenum graecum* (fenugreek) have been reported to have antidiabetic and hypocholesterolaemic properties in both animal models and humans (Hannan *et al*., 2003) Activity has been attributed largely to fenugreek's saponin and high fiber and unsaturated/ saturated fatty acid ratio contents of the seeds, and is probably not related to its major alkaloid trigonelline. Fenugreek administration may increase plasma insulin levels in vivo. The hypocholesterolaemic effect has been attributed to the increased conversion of hepatic cholesterol to bile salts which are lost in the faces together with fenugreek fiber and saponins. Fenugreek treatment selectively reduces the LDL-c and increases HDL-c (Molham and Amala, 1998).

1.2.3. Fibers:

Fibrates (including bezafibrate, gemfibrozil and fenofibrate) are a group of lipid lowering drugs that have been in existence for over 40 years. They are usually used in patients with mixed or combined hyperlipidemia and hypertriglyceridemias (Winston, 1999). Fibrates are reported to decrease plasma triglycerides by decreasing their hepatic synthesis and increasing their catabolism. They decrease the triglyceride- VLDL synthesis through enhancing beta-oxidation of fatty acids in the liver and increase the plasma triglycerides catabolism by inducing lipoprotein lipase gene transcription and decreasing the apoC-III gene transcription. Fibrates are reported to increase HDL-c by increasing apoA-I and apoA-II gene transcription (Duriez, 2003).

1.3 General taxonomy of Trigonella foenum-graecum:

Vernacular name (Ar): Hulabah

1.3.1 Scientific classification:

Kingdom: planate

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: *Trigonela*

Species: *T. foenum-graecum*

1.3.2 Folkloric uses of fenugreek:

Fenugreek (*Trigonella foenum graecum L.*) is one of the oldest medicinal plants, originating in India and Northern Africa. An annual plant, fenugreek grows to an average height of two feet. The leaves and seeds, which mature in long pods, are used to prepare extracts or powders for medicinal use. Applications of fenugreek were documented in ancient Egypt where it was used in incense and to embalm mummies. In modern Egypt, fenugreek is still used as a supplement in wheat and maize flour for bread-making (Morcos *et al.*, 1981). In ancient Rome, fenugreek was purportedly used to aid labor and delivery. In traditional Chinese medicine, fenugreek seeds are used as a tonic, as well as a treatment for weakness and edema of the legs (Yoshikawa *et al.*, 1997). In India, fenugreek is commonly consumed as a condiment and used medicinally as a lactation stimulant. There are numerous other folkloric uses of fenugreek, including the treatment of indigestion and baldness. (Patil *et al.*, 1997).

1.3.3 Chemical composition of Fenugreek:

Chemical analysis of fenugreek indicates that the seeds are a rich source of protein, unavailable carbohydrate, mucilages and saponins (El-Mahdy and El-

Sebaiy, 1985). Fenugreek resembles guar gum in its high dietary fibre content, and its high viscosity (Valette *et al*., 1984). Fenugreek seeds are also rich in saponins (Sharma, 1986). Anis and Aminuddin. (1985) reported the presence of three steroidal sapogenins, diosgenin, gitogenin and tigogenin. The use of more sophisticated analytical techniques including coupled GC-MS have detected and identified ten different sapogenins (Brenac and Sauvaire, 1996). The presence of a sapogenin peptide ester, fenugreekine has been reported (Ghosal *et al*., 1974). Some of the biological properties of the purified steroid saponins have been evaluated (Sauvaire *et al*., 1996) and include hypocholesterolaemic and antifungal activity as well as effects on food intake, feeding behavior and motivation in rats (Petit et al., 1995). Except for differences in fat and saponin content, fenugreek seed powder and the defatted fenugreek are chemically similar, containing almost equal amounts of amino acids, minerals and vitamins. Fenugreek, like other legumes, is rich in arginine, alanine and glycine; but poor in lysine content (Sharma, 1984).

In the seeds like other legumes, the protein is deficient in methionine. Trigonelline is an important alkaloidal component of the seeds. The seeds contain less starch but higher proportions of minerals (Ca, P, Fe, Zn and Mn) compared with other grain legumes (Sankara and Deosthale, 1981). The total lipid content (7.5%) of the seeds consists of neutral lipids, glycolipids and phospholipids (Hemavathy and Prabhakar, 1989). The aromatic constituents of the seeds have been elucidated (Girardon *et al*., 1985) and include n-alkanes, sesquiterpenes and some oxygenated compounds such as hexanol and g- nonalactone. The seeds are also known to contain flavonoids, carotenoids, coumarins and other components (Varshney and Sharma, 1996).

CHAPTER TWO
MATERIAL AND METHODS

2.1. Experimental details:

This experiment was designed to evaluate the effect of feeding *Trigonella foenum graecum* seed powder (TSP) mixed diet on plasma lipid concentration in an induced hypercholesterolemic Wistar albino rats.

2.1.1. Experimental animals:

Twenty Wistar albino rats obtained from the National Center for Research, Khartoum were used in this study. The rats were housed identically in stainless steel cages in an air room under suitable conditions. All of the rats were initially fed a standard laboratory diet for at least 7 days to acclimatize to our laboratory. Tap water was freely available.

2.1.2. The rat basal diet:

The rats were given a basal diet which fulfilled their requirement. The composition was as follows:

Wheat flour	692g
Dry meat	165g
Sodium chloride	3g
Oil	120g

2.2. Plant material:

The fenugreek seeds was locally purchased, identified and peeled in fine pieces then added to the diet.

2.3. Cholesterol supplementation in the diet:

1% cholesterol powder was supplemented to the basal diet of the rats so as to induce hypercholesterolemia in all groups except the control group, according to (Sharma, 1984).

2.4. Equipment used:

- Heparinized capillary tubes.
- Heparinized blood containers.
- Plane containers.
- Centrifuge.
- Automatic pipettes.
- Roche diagnostic/Hitachi 902 analyzer.

2.4.1. Chemicals:

- Cholesterol powder.

2.5. Experimental procedure:

The animals were divided into four groups of five animals each. These groups were named as A, B, C, and D. Group A was given the basal diet and served as control, group B received 1% cholesterol added to the basal diet. Group C received 1% cholesterol and 4% Trigonella foenum graecum seed powder TSP added to the basal diet, Group D received 1% cholesterol and 8% TSP added to the basal diet. Blood samples were collected after two weeks following treatment so as to confirm the induction of hypercholesterolemia. Then after another two weeks the blood samples were taken for the determination of lipid fractions concentration.

2.5.1. Blood sampling:

1.5 ml of blood was collected from the rats orbital plexus after an overnight fast by capillary tubes and was put in heparinized containers; the blood was centrifuged at 5000 rpm for 10 minutes. Then the plasma was placed into plane containers and used immediately.

2.6. Analytical methods:

2.6.1. Roche diagnostic/Hitachi 902 analyzer:

All lipid fractions were estimated using the Roche diagnostic Hitachi 902 analyzer. It is an analyzer to report test results on various body fluid samples for wide range of analysis. It is fully automated, computerized, performs potentiometric and photometric assays, and includes analytical processing unit and luminance crystal display (LCD) touch screen, with a standard printer to print the results. The analyzer is characterized by doing 200 photometric tests/ hour, and refrigerated storage for 40 reagent containers, as well as it has end point, and kinetic and isoenzymes reactions.

2.6.2. Total cholesterol estimation:

Principle:

In the presence of cholesterol esterase, the cholesterol esters in the sample are hydrolyzed to cholesterol and free fatty acids. The cholesterol produced is oxidized by cholesterol oxidase to cholesterol and hydrogen peroxide. Hydrogen peroxide is detected by chromogenic oxygen acceptor, phenol – ampyrone, in the presence of peroxidase. The red quinine formed is proportional to the amount of cholesterol present in the sample.

Reagents and linearity:

Reagent (1)	buffer pH 6.9	90 mmol/L
	Phenol	26 mmol/L
Reagent (2)	Cholesterol esterase	300 U/L
	Cholesterol oxidase	300 U/L
	Phenol- ampyrone/ peroxidase	1250 U/L
	4 – Aminoantipyrine	0.4 mmol/L

Linearity range: This method is linear up to 600 mg/dl

Procedure:

The analyzer automatically adds an equal quantity from sample, R_1 and R_2 to the reaction.

Calculations:

The analyzer automatically calculates the analyte concentration of each sample. The result will appear directly on the screen of the diagnostic machine at the end of the reaction between the reagents and samples.

2.6.3. Low density lipoprotein-cholesterol (LDL-c) estimation:

Principle:

LDL-c particles in the sample are precipitated with polyvinyl sulphate. Their concentration is calculated from the difference between the serum total cholesterol and the cholesterol in the suprnatant after centrifugation. The cholesterol is spectrophometrically measured by means of the coupled reactions as described for cholesterol.

Reagents and linearity:

Reagent (1): polyvinyl sulphate 3g/L, polyethylene glycol 3g/L.

Procedure:

The analyzer automatically adds an equal quantity from sample and reagents to the reaction.

Calculations:

The analyzer automatically calculates the analyte concentration of each sample. The result will appear directly on the screen of the diagnostic machine at the end of the reaction between the reagents and samples.

2.6.4. High density lipoprotein-cholesterol (HDL-c) estimation:

Principle:

Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the sample are precipitated with phosphotungstate and magnesium ions. The supernatant contains HDL-c. The HDL-c is then spectrophotometrically measured by means of coupled reactions described for cholesterol.

Reagents:

Reagent (B). 50 ml phosphotungstate (0.4 m mol/L) and magnesium (chloride 20 m mol/L).

HDL-c standard concentration = 40 mg/dl.

Linear Range: this method is linear up to 600 mg/dl.

Procedure:

The analyzer automatically adds an equal quantity from the sample and reagents to the reaction.

Calculations:

The analyzer automatically calculates the analyte concentration of each sample. The result will appear directly on the screen of the diagnostic machine at the end of the reaction between the reagents and samples.

2.6.5. Triglycerides estimation:

Principle:

Triglycerides in the sample are hydrolyzed enzymatically to glycerol and fatty acids. The glycerol formed is converted to glycerol phosphate by glycerol kinase. Glycerol phosphate is oxidized to dihydroxyacetone phosphate by glycerol phosphate oxidase. The liberated hydrogen peroxide is detected by a chromogenic acceptor, chlorophenol and 4-amino antipyrine in the presence of peroxidase. The red quinone formed is proportional to the amount of triglyceride present in the sample.

Reagents and linearity:

Reagent (1)	Buffer	45 mmol/L
	Chlorophenol	6 mmol/L
Reagent (2)	Magnesium chloride	5 mmol/L
	Lipase	>100 u/ MI
	Glycerol kinase	>1.5 u/mL
	Glycerol -3- oxidase phosphate	>4 u/ mL
	Peroxidase	>0.8 u/mL
	4- aminoantipyrine	0.75 mmol/L
	ATP	0.9 mmol/L
	PH	7.5

Linearity range: This method is linear up to 600 mg/dl.

Procedure:

The analyzer automatically adds an equal quantity from sample, R_1 and R_2 to the reaction.

Calculations:

The analyzer automatically calculates the analyte concentration of each sample. The result will appear directly on the screen of the diagnostic machine at the end of the reaction between the reagents and samples.

Quality Control:

The precision and accuracy of all methods used in this study were checked each time a batch was analyzed by including commercially prepared control sera.

2.6.6. Statistical analysis:

Results were analyzed using SPSS version 11 analysis of variances one way ANOVA. The significance was determined at 5% level by using the t-test.

CHAPTER THREE

RESULTS

3.1. The induction of hypercholesterolemia:

There is a significant increase in the total cholesterol level as well as LDL-c, and a significant decrease in HDL-c in group B compared to the control group. These results confirm the induction of hypercholesterolemia in group B as shown in table (1) and Fig. (1, 2 and 3).

Table (1): The levels of total cholesterol, LDL-c and HDL-c in group B compared to group A two weeks after supplementation of 1% cholesterol.

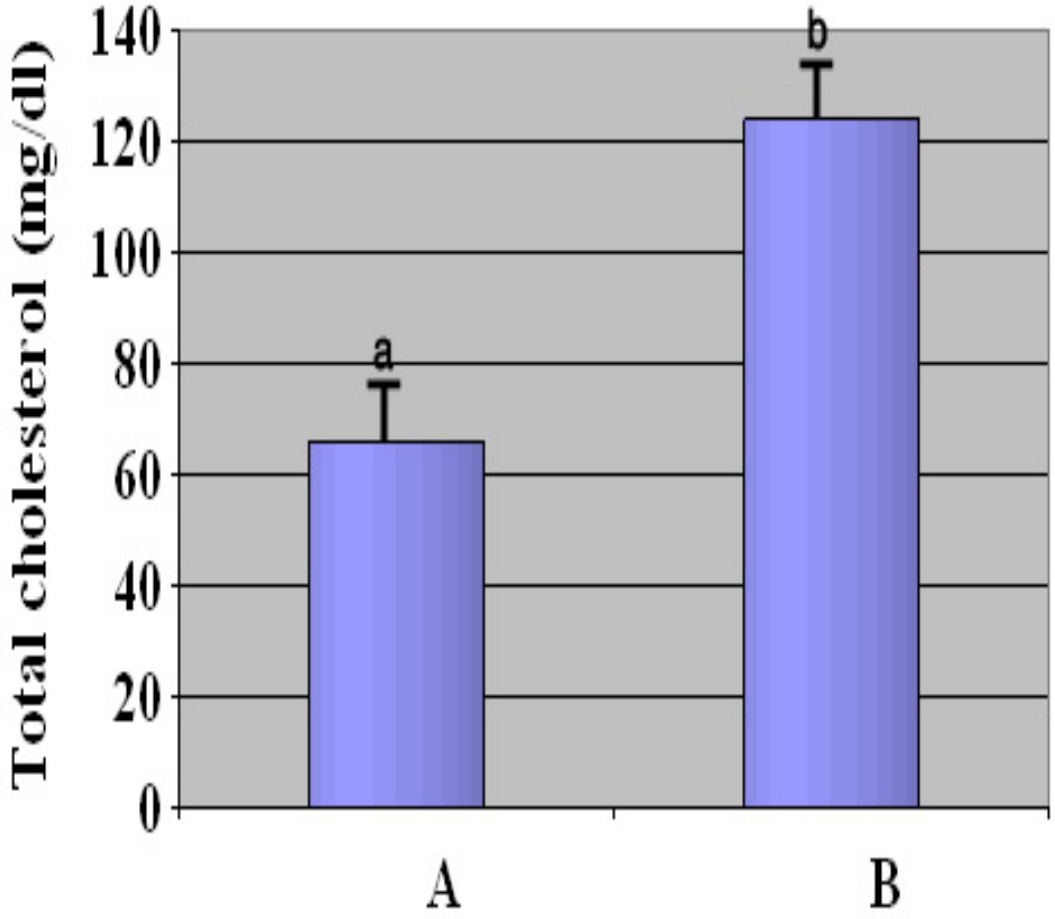
Parameters / Groups	T-cholesterol (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
A	66.00±9.71[a]	12.58±3.99[a]	50.24±7.95[a]
B	124.60±8.17[b]	58.34± 5.68[b]	38.20±3.56[b]

Means ± SE within the same column having different superscript small letters are significantly different at (P < 0.05) based on t- test.

Group A: Fed basal diet and served as control.

Group B: Fed 1% cholesterol mixed with the basal diet.

Fig. (1): The level of total cholesterol in group B compared to group A two weeks after supplementation of 1% cholesterol.

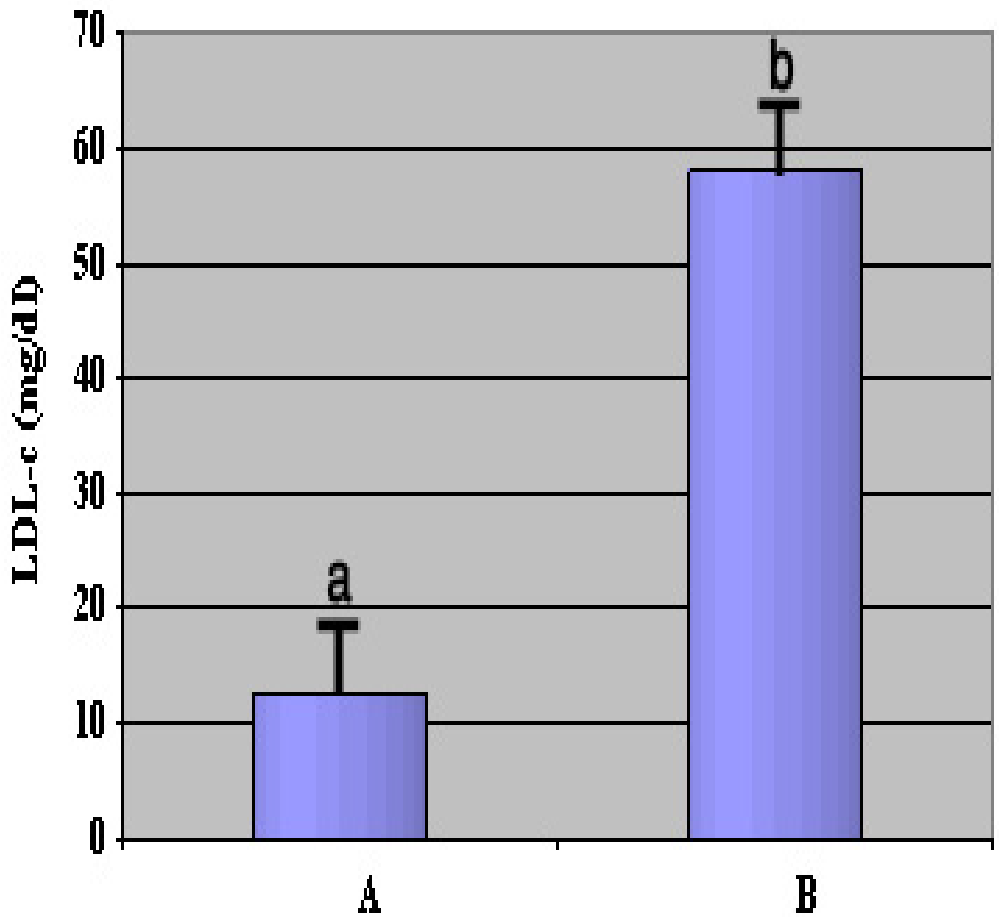


Bars having different superscript small letters are significantly different at (P < 0.05) based on t- test.

Group A: Fed basal diet and served as control.

Group B: Fed 1% cholesterol mixed with the basal diet.

Fig. (2): The level of LDL-c in group B compared to group A two weeks after supplementation of 1% cholesterol.

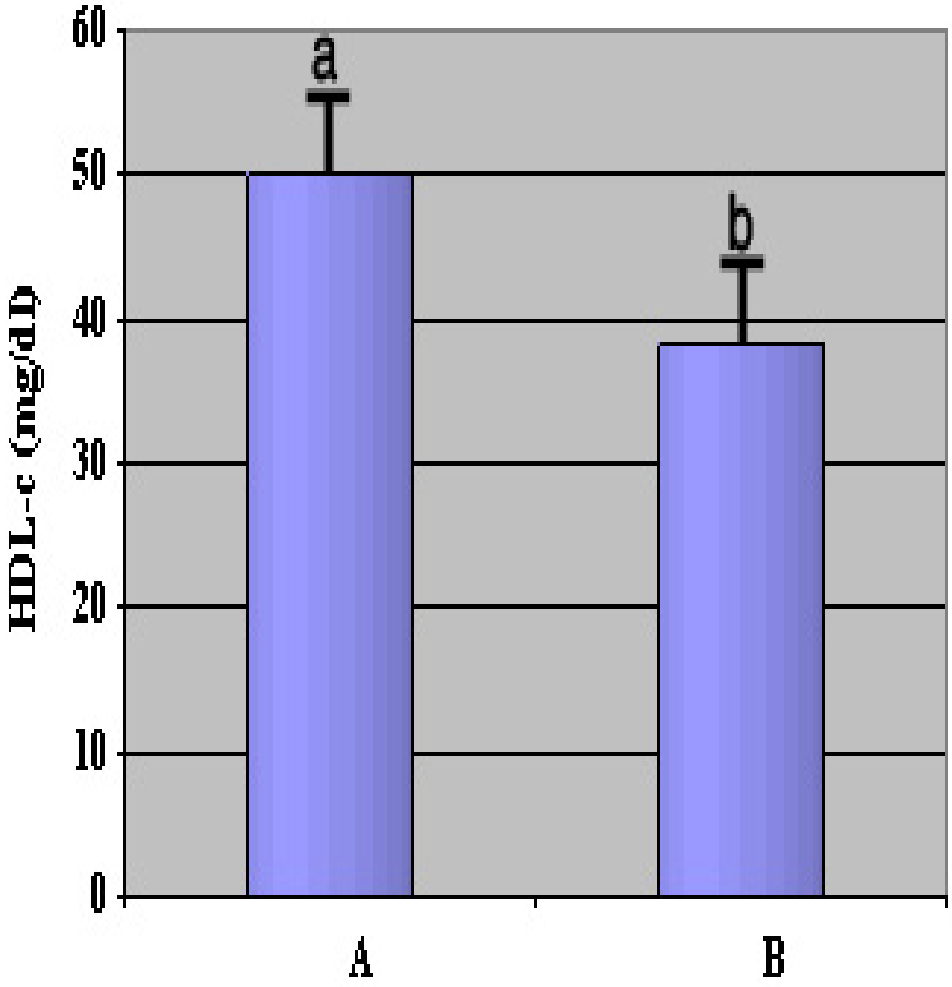


Bars having different superscript small letters are significantly different at ($P < 0.05$) based on t- test.

Group A: Fed basal diet and served as control.

Group B: Fed 1% cholesterol mixed with the basal diet.

Fig. (3): The level of HDL-c in group B compared to group A two weeks after supplementation of 1% cholesterol.



Bars having different superscript small letters are significantly different at (P < 0.05) based on t- test.

Group A: Fed basal diet and served as control.

Group B: Fed 1% cholesterol mixed with the basal diet.

3.2. The effect of feeding fenugreek seeds powder on plasma total cholesterol level in an induced hypercholesterolemic Wistar albino rats:

Table (2) and Fig. (4) show the results of plasma total cholesterol of group A, B, C and D. The level of plasma total cholesterol in group B is significantly ($P< 0.05$) higher than the levels of plasma total cholesterol in group A, C and D. in group C the level of plasma total cholesterol is non-significantly higher than that of group A and group D, and significantly ($P < 0.05$) lower than the level of plasma total cholesterol in group B. However, in group D the level of plasma total cholesterol is significantly ($P < 0.05$) lower than the level of plasma total cholesterol in group B but non- significantly different form group A and group C.

3.3. The effect of feeding fenugreek seeds powder on plasma LDL-c level in an induced hypercholesterolemic Wistar albino rats:

Table (2) and Fig. (5) show the results of plasma LDL- c of group A, B, C and D. The level of plasma LDL-c in group B is significantly ($P< 0.05$) higher than the levels of plasma LDL-c in group A, C and D. in group C the level of plasma LDL-c is non-significantly different compared to the levels of plasma LDL-c of group A and group D, and significantly ($P < 0.05$) lower than the level of plasma LDL-c in group B. However, in group D the level of plasma LDL-c is significantly ($P < 0.05$) lower than the level of plasma LDL-c in group B but non- significantly different form groups A and group C.

Table (2): The effect of feeding fenugreek seeds powder on plasma total cholesterol, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol and triglycerides level in an induced hypercholesterolemic Wister albino rats:

Parameters / Groups	T-cholesterol (mg/ dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	Triglycerides (mg/dl)
A	**84.00 ±1.87^{a}**	**2.58±3.99^{a}**	**52.60±6.45^{a}**	**86.00±3.48^{a}**
B	**121.67±11.59^{b}**	**58.34±5.68^{b}**	**38.40±2.00^{b}**	**86.25 ±6.31^{a}**
C	**91.41±3.10^{a}**	**20.60±1.28^{a}**	**57.40±5.27^{a}**	**83.33 ±2.02^{a}**
D	**90.25±3.47^{a}**	**23.28±2.99^{a}**	**57.38±2.27^{a}**	**70.67 ±9.17^{a}**

Means ± SE within the same column having different superscript small letters are significantly different at (P < 0.05) based on t- test.

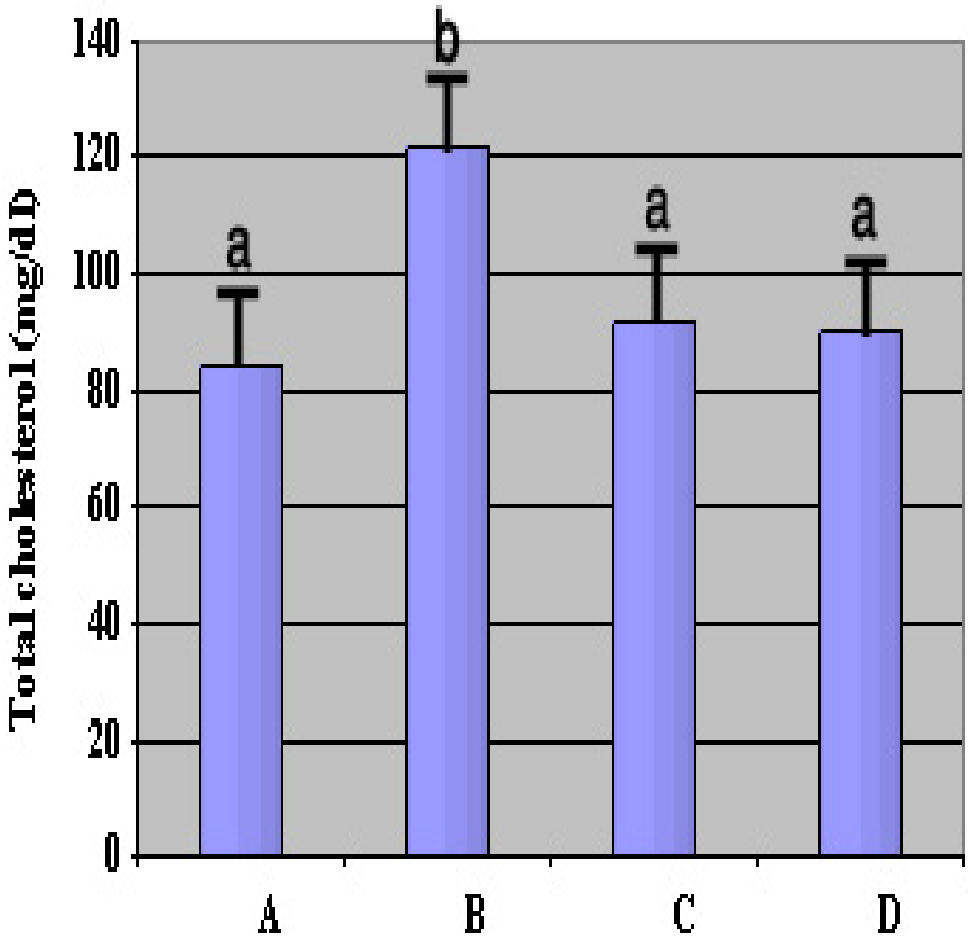
Group A: fed basal diet.

Group B: fed 1% cholesterol basal diet.

Group C: fed 1% cholesterol and 4% TSP added to the basal diet.

Group D: fed 1% cholesterol and 8% TSP added to the basal diet.

Fig. (4): The effect of feeding fenugreek seeds powder on plasma level of total cholesterol in an induced hypercholesterolemic Wistar albino rats.



Bars having different superscript small letters are significantly different at (P < 0.05) based on t- test.

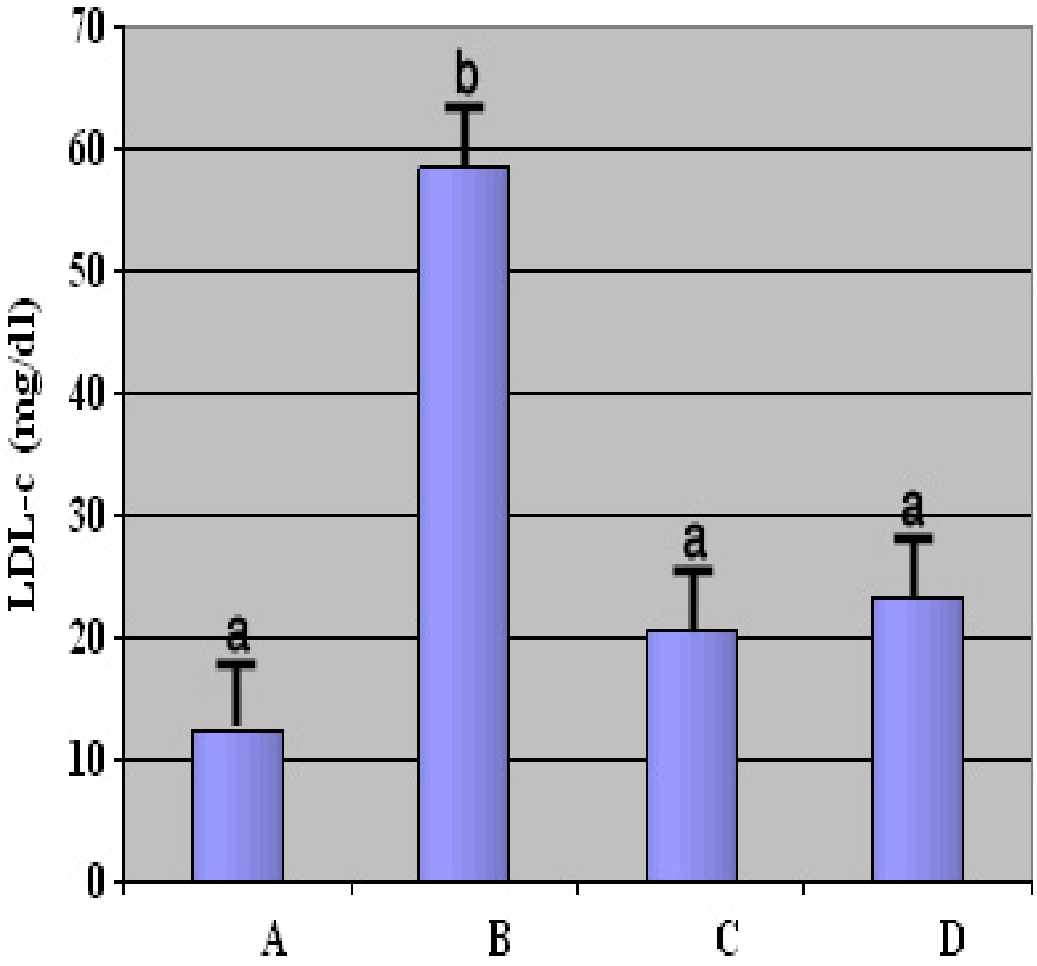
Group A: Fed basal diet.

Group B: Fed 1% cholesterol basal added to the diet.

Group C: Fed 1% cholesterol and 4% TSP added to the basal diet.

Group D: Fed 1% cholesterol and 8% TSP added to the basal diet.

Fig. (5): The effect of feeding fenugreek seeds powder on plasma level of LDL-c in an induced hypercholesterolemic Wistar albino rats.



Bars having different superscript small letters are significantly different at (P < 0.05) based on t- test.

Group A: Fed basal added diet.

Group B: Fed 1% cholesterol added the basal diet.

Group C: Fed 1% cholesterol and 4% TSP added to the basal diet.

Group D: Fed 1% cholesterol and 8% TSP added to the basal diet.

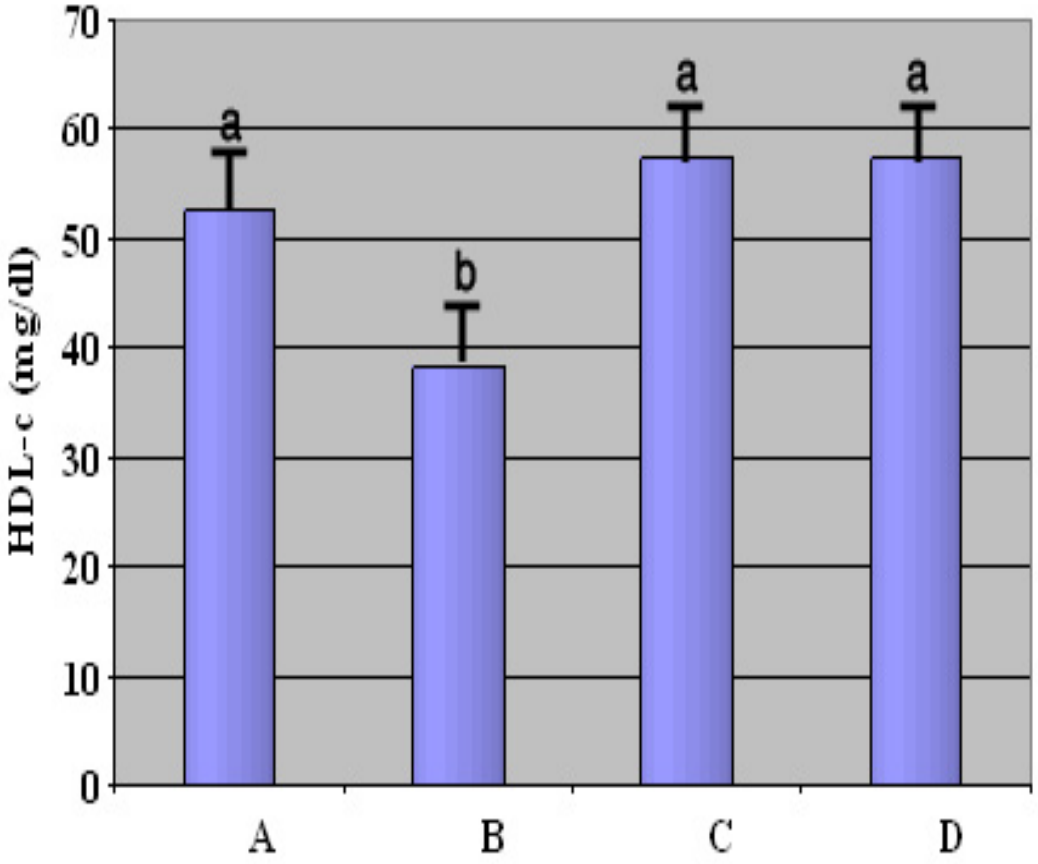
3.4 The effect of feeding fenugreek seeds powder on plasma HDL-c level in an induced hypercholesterolemic Wistar albino rats:

Table (2) and Fig. (6) show the results of plasma HDL-c of groups A, B, C and D. The level of plasma HDL-c in group B is significantly ($P< 0.05$) lower than the levels of plasma HDL-c in group A, C and D. In group C the level of plasma HDL-c is non-significantly different compared to the levels of plasma HDL-c of group A and group D, and significantly ($P < 0.05$) higher than the level of plasma HDL-c in group B. However, in group D the level of plasma HDL-c is significantly ($P < 0.05$) lower than the level of plasma HDL-c in group B but non- significantly different form group A and group C.

3.5 The effect of feeding fenugreek seeds powder on plasma triglycerides level in an induced hypercholesterolemic Wistar albino rats:

Table (2) and Fig. (7) show the results of plasma triglycerides level of group A, B, C and D. The level of plasma triglycerides in group B is non-significantly higher than the levels of plasma triglycerides in group A, C and D. in group C the level of plasma triglycerides is non-significantly lower than the levels of plasma triglycerides of group A and group B, and non-significantly higher than the level of plasma triglycerides in group D. However, in group D the level of plasma triglycerides is non-significantly lower than the level of plasma triglycerides in group A, B, and C.

Fig. (6): The effect of feeding fenugreek seeds powder on plasma level of HDL-c in an induced hypercholesterolemic Wistar albino rats.



Bras having different superscript small letters are significantly different at ($P < 0.05$) based on t- test.

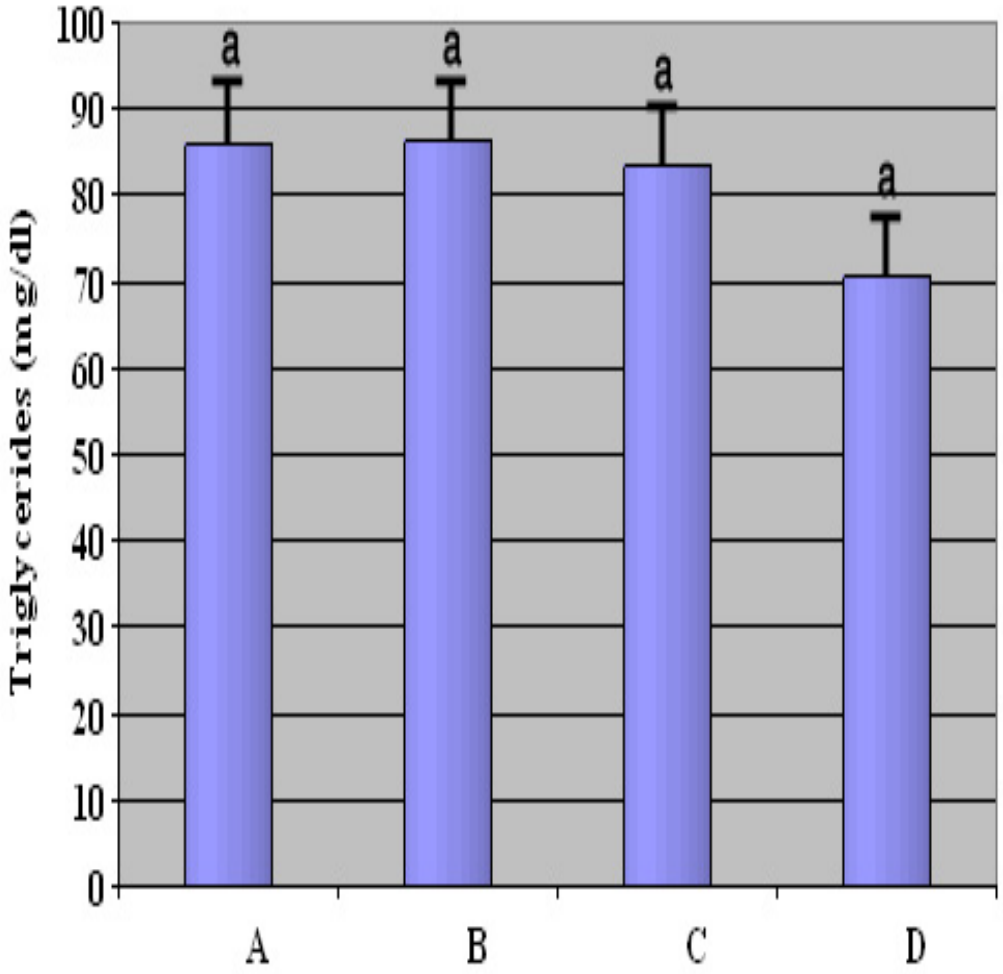
Group A: fed basal diet.

Group B: fed 1% cholesterol added to the basal diet.

Group C: fed 1% cholesterol and 4% TSP added to the basal diet.

Group D: fed 1% cholesterol and 8% TSP added to the basal diet.

Fig. (7): The effect of feeding fenugreek seeds powder on plasma level of triglycerides in an induced hypercholesterolemia Wistar albino rats.



Bars having different superscript small letters are significantly different at (P < 0.05) based on t- test.

Group A: fed basal diet.

Group B: fed 1% cholesterol added to the basal diet.

Group C: fed 1% cholesterol and 4% TSP added to the basal diet.

Group D: fed 1% cholesterol and 8% TSP added to the basal diet.

CHAPTER FOUR
DISSCUSION

This study was carried out to evaluate the effect of feeding *Trigonella foenum graecum* seeds powder (TSP) on plasma levels of total cholesterol, low density lipoprotein-cholesterol (LDL-c), high density lipoprotein-cholesterol (HDL-c) and triglycerides in an induced hypercholesterolemic Wistar albino rats.

4.1 Induction of hypercholesterolemia:

The results showed that the plasma total cholesterol and LDL-c levels were significantly increased ($P< 0.05$) following administration of 1% cholesterol powder mixed with the basal diet after two weeks in group B compared to group A (control), but there was a significant ($P< 0.05$) decrease in HDL-c level. These results were in agreement with the study of Sharma (1984), who reported that administration of 1% cholesterol powder resulted in an increase total and LDL-c, but the HDL-c was reported to be decreased. Also the results were in line with the result obtained by Shela *et al*. (2005) who reported that administration of 1% cholesterol powder to rabbits resulted in an increase total and LDL-c, but the HDL-c was reported to be decreased.

4.2 The effect of feeding fenugreek seeds powder on the levels of:

4.2.1 Plasma total cholesterol:

The level of plasma total cholesterol was decreased significantly ($P<0.05$) after the administration of 4% and 8% TSP compared to the control. These results were in line with those obtained by Yadav *et al.* (2004) who reported that feeding 5% of TSP mixed with the powdered rat feed for 21 days resulted in a significant reduction of serum total cholesterol, this is may be due to the high fiber content of TSP. The results were also in line with the results stated by Molham and Amala (1998), who reported that feeding of 50% TSP mixed diet for two weeks to hypercholesterolemic rats resulted in a reduction of Plasma total cholesterol, which is attributed to an increased conversion of hepatic cholesterol to bile salts which is lost in the faces together with fenugreek fiber and saponins. Also the TSP improves insulin secretion, which has an inhibitory action on

HMG-CoA reductase, a key rate limiting enzyme responsible for the synthesis of cholesterol (Petit *et al*., 1995).

Hannan *et al*. (2003) reported that the administration of the soluble dietary fiber (SDF) fraction of *Trigonella foenum graecum* (Tf-sdf) to rats orally twice daily at a dose of 0.5 g kg^{-1} for 28 days lowered the serum cholesterol level. Tf-sdf fraction effectively inhibits fats absorption in the gut (Madar and Shomer, 1990). Therefore, the hypolipidemic action of the soluble fraction could be as a result of impairment of fats absorption due to the presence of high amounts of fiber in the seeds.

According to the above mentioned results the hypocholesterolaemic effect of TSP may be due to impairment of fats absorption due to the presence of fiber in the seeds and increased conversion of hepatic cholesterol to bile salts which is lost in the faces together with fenugreek fiber and saponins. Also the hypocholesterolemic effect of TSP may be due to the improvement of insulin secretion because insulin has an inhibitory action on HMG-CoA reductase, a key rate limiting enzyme responsible for the synthesis of cholesterol.

4.2.2 Plasma LDL-c:

The level of Plasma LDL-c decreased significantly ($P<0.05$) after the administration of 4% and 8% TSP. These results were in line with the results stated by Molham and Amala (1998), who reported that feeding of 50% TSP mixed diet for two weeks to hypercholesterolemic rats resulted in a reduction of Plasma LDL-c, which may be due to the high fiber and saponins contents of the TSP. Hwang *et al*. (2001) reported that feeding of 5% sativum whole fruits to rats after high fat diet decreased LDL-c level significantly due to the fiber content that increases LDL-c receptor activity.

The results were also agreement with those obtained by Sharma *et al*. (1996) who reported that feeding 10% TSP mixed with rats basal diet for two weeks resulted in a significant decrease of plasma LDL-c level which is explained to be due to the high fiber content of the seeds.

From the above mentioned results the reduction of LDL-c level by TSP is due to the high fiber and saponins contents of the TSP which increases LDL-c

receptors activity that enhance the uptake of LDL-c particles by cells. Also the fiber and saponins contents of the TSP reduce the release of LDL-c particles from the hepatocytes.

4.2.3 Plasma HDL-c:

In the present study addition of 4% and 8% of TSP to the basal diet of an induced hypercholesterolemic Wistar albino rats resulted in a significant increase of Plasma HDL-c. These results agree with the results stated by Molham and Amala (1998), who reported that feeding of 50% TSP mixed diet for two weeks to hypercholesterolemic rats resulted in the elevation of Plasma HDL-c level, which is attributed to the high fiber and unsaturated/ saturated fatty acids ratio contents of the seeds.

Hannan *et al*. (2003) reported that the administration of the soluble dietary fiber (SDF) fraction of *Trigonella foenum graecum* (Tf-sdf) to rats orally twice daily at a dose of 0.5 g kg^{-1} for 28 days increased the serum HDL-c level. Tf-sdf fraction effectively inhibits fat absorption due to the presence of high fiber concentration in the seeds this lead to decrease VLDL production and increase HDL-c level.

According to the above mentioned findings the elevated level of HDL-c is attributed to the high fiber and unsaturated/ saturated fatty acids ratio contents of the seeds which decrease VLDL and increase HDL-c production.

4.2.4 Plasma triglycerides:

In the present study addition of 4% and 8% of TSP to the basal diet of an induced hypercholesterolemic Wistar albino rats resulted in a non significant decease of plasma triglycerides level. These findings were in line with Sowmya and Rajyalakshmi (1999), who observed a non- significant reduction in plasma triglycerides level in 20 adult individuals with hypercholesterolemia who received 12.5-18.0 g powdered fenugreek seeds for one month. But these results disagree with Alam *et al*. (2003) who concluded that feeding of 1g, 3g and 6g cinnamon (contain the same hypolipidemic agents that present in fenugreek) per day to type 2 diabetic individuals for 60 days reduced significantly triglycerides concentration. This may be due to the short duration of this experiment.

From the above mentioned findings the TSP may need more than one month to display it's hypotriglceridemic effect.

CONCLUSION:

The results indicated that mixing of *Trigonella foenum graecum* seeds powder (TSP) with diet significantly reduced atherogenic lipids in Wistar albino rats when given at doses of 4% and 8% for one month.

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